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Differentiation of *Coronocyclus sagittatus* and *Coronocyclus coronatus* (Nematoda: Cyathostominea) of Horses

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ABSTRACT: An increased interest worldwide in small strongyles of horses has prompted a revision of the systematics in order to support research on improving antiparasitic chemotherapy, biocontrol, and other management practices. A 1997 international workshop developed a revised list of recommended names for 51 recognized species of Cyathostominea and indicated problems in the differentation of some species, including *Coronocyclus coronatus* (Looss, 1900) and *C. sagittatus* (Kotlan, 1920). These morphologically similar species are members of *Coronocyclus* Hartwich, 1986, a genus with prominent and sclerotized external leaf crown supports that are separated from the buccal capsule. The two species can be distinguished from one another by differences in the shape of the buccal capsule, in the shape of the buccal capsule wall in optical section, in the point of insertion of elements of the internal leaf crown, and in the dorsal ray of the male. Drawings and photomicrographs are presented that illustrate the characteristics useful for differentiating *C. coronatus* and *C. sagittatus*.

KEY WORDS: *Coronocyclus sagittatus, Coronocyclus coronatus,* Nematoda, Cyathostominea, horses.

The Cyathostominea, or small strongyles of horses, can cause considerable morbidity and mortality in horses (Herd, 1990). Research activity on these nematodes is currently high for a number of reasons: 1) there is increasing recognition of larval cyathostominosis, a syndrome in which large numbers of larvae emerge from the walls of the large intestine, colon, and cecum, causing severe colitis that may result in death (Mair, 1994; van Loon et al., 1995); 2) resistance to anthelmintics in the Cyathostominea has been widely reported (Herd and Coles, 1995; Ihler, 1995); and 3) biological control prospects using nematode-trapping fungi appear promising (Bird and Herd, 1995; Larsen et al., 1996). This increased research interest required an update of the taxonomy and systematics of the Cyathostominea; the update was based on 3 differing classifications of genera and species (Lichtenfels, 1975; Hartwich, 1986; Dvojnos and Kharchenko, 1994). Differences among these classifications were resolved at an international workshop in Sun City, South Africa (Lichtenfels et al., 1998). The workshop developed a revised list of recommended names for 51 recognized species of Cyathostominea and indicated problems in the differentiation of some species, including difficulties in separating 2 similar species of the genus *Coronocyclus* Hartwich, 1986, *C. coronatus* (Looss, 1900) and *C. sagittatus* (Kotlan, 1920). The objective of this report is to describe morphological characteristics that can be used to differentiate these 2 species.

The genus *Coronocyclus* was defined (Hartwich, 1986) by distinguishing from the genus *Cyathostomum* (sensu Lichtenfels, 1975) the 4 species that have the sclerotized support of the external leaf crown (ELC) separated anteriorly from the wall of the buccal capsule. A fifth species was added to the genus by Dvojnos et al. (1994). *Coronocyclus* was recognized by the Sun City workshop as having the following 5 species: (1) *C. coronatus* (Looss, 1900) Hartwich, 1986, type species, =*Trichonema subcoronatum* Yamaguti, 1943; (2) *C. labiatus* (Looss, 1902) Hartwich, 1986, =*Cylicostomum labiatum digitatum* Ihler, 1921; (3) *C. labratus* (Looss, 1900) Hartwich, 1986; (4) *C. sagittatus* (Kotlan, 1920) Hartwich, 1986; and (5) *C. ulambajari* Dvojnos, Kharchenko, and Lichtenfels, 1994. Of these 5 species, only *C. sagittatus* and *C. coron-*

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Table 1. Geographic locality, host, number, sex, and type of specimens of *Coronocyclus sagittatus* and *C. coronatus* studied.

Geographic locality	Host	USNPC No.	No. studied		Type	Collector
			Males	Females		
Coronocyclus sagittatus						
Japan	*Equus caballus*	79081	2	2	Voucher	M. Ito
Mongolia	*Equus caballus*	83402		4	Voucher	G. M. Dvojnos
Moscow, Russia	*Equus caballus*	83403	2	1	Voucher	G. M. Dvojnos
Coronocyclus coronatus						
Cairo, Egypt	*Equus caballus*	9598	2	2	Syntypes	A. Looss
Panama	*Equus caballus*	58476	2	2	Voucher	A. O. Foster
Frederiksberg, Denmark	*Equus caballus*	85073	2	2	Voucher	J. Monrad

atus are difficult to distinguish from each other. In fact, Skladnik (1935) considered *C. sagittatus* to be a synonym of *C. coronatus*. However, Ershov (1933) and most, if not all, modern researchers recognize both species (McIntosh, 1951; Popova, 1958; Baruš, 1962; Lichtenfels, 1975; Hartwich, 1986; Dvojnos and Kharchenko, 1994). *Coronocyclus sagittatus* has been reported only rarely from Eastern Europe and Asia and once from the Caribbean islands (Hartwich, 1994). Because it has not been collected from North America, it was described only briefly in the most widely available identification manual (Lichtenfels, 1975). With the international movement of horses, *C. sagittatus* can be expected to have a wider distribution than has been indicated in the literature. Hartwich (1994) suggested that the distribution of *C. sagittatus* is probably more extensive than has been reported because of the difficulty in distinguishing it from the similar species *C. coronatus*. The information in this article will enable *C. sagittatus* to be identified and separated from the commonly reported species *C. coronatus*.

Materials and Methods

Type specimens of *C. coronatus* were obtained from the U.S. National Parasite Collection (USNPC) maintained by the Agricultural Research Service at Beltsville, Maryland. Other specimens studied are listed in Table 1. Scientific naming follows the recommendations of the 1997 Workshop on the systematics of the Cyathostominea reported in an annotated checklist by Lichtenfels et al. (1998). Nematodes were studied in temporary wet mounts in the clearing agent phenol-alcohol (80 parts melted phenol crystals in 20 parts absolute ethanol) with the aid of a light microscope equipped with interference contrast optics.

Drawings were made with the aid of a camera lucida attached to the microscope; these drawings have been published previously (Dvojnos and Kharchenko, 1994). Photomicrographs were obtained with a 35-mm camera mounted on an Olympus Vanox research microscope at magnifications ranging from ×100–400 using Kodak T-Max black-and-white negative film.

Measurements are in micrometers unless otherwise indicated (Tables 2, 3). Measurements employed standard methods. Buccal capsule depth was measured from the anterior edge of the buccal capsule wall to the anterior edge of the esophagus. Buccal capsule width was measured from the outside edges at its widest point. Measurements of previous authors are included in Tables 2 and 3 for comparison.

The term "anterior deirid" is used for the bilateral cervical papillae, even though the posterior deirid described in other nematodes (Lichtenfels et al. 1995) of the class Secernentea has not been described in the Cyathostominea.

Results

The 2 similar species *Coronocyclus coronatus* and *C. sagittatus* can be distinguished from one another by differences in the shape of the buccal capsule wall, in the point of insertion of elements of the internal leaf crown (ILC), and in the configuration and spacing of the branches of the dorsal bursal ray of the male copulatory bursa.

SHAPE OF BUCCAL CAPSULE: The buccal capsule of both species is nearly cylindrical, and it is somewhat wider than deep. The capsule of *C. sagittatus* is proportionally wider than that of *C. coronatus* (Figs. 1, 2, 11, 12, 19–22; Tables 2, 3).

SHAPE OF BUCCAL CAPSULE WALL IN OPTICAL SECTION: The shape of the buccal capsule wall in the optical section is thumblike, thicker at the bottom and middle than at the top. In *C. sagittatus,* the anterior portion of the wall is thinner, especially in a lateral view, than it is in *C. co-*

Table 2. Morphometric comparison (in micrometers unless otherwise indicated, range and mean) of *Coronocyclus sagitttatus* with data reported by Kotlan (1920),* Dvojnos and Kharchenko (1994), and Hartwich (1994).

Characters	Present study	Kotlan (1920)	Dvojnos and Kharchenko (1994)	Hartwich (1994)
Males				
Body length (mm)	8.14–11.3 (9.93)	10.0–11.0	9.50–11.0	9.70–11.0
Body width	375–492 (448)	400	—	400–520
Buccal capsule width	116–135 (124)	100–120	146–160	72–113
Buccal capsule depth	41–49 (45)	40	32–36	32–50
Elements of ELC	—	18–20	16–20	16–20 (18)
Elements of ILC	—	70–80	60–80	66–74
Nerve ring†	307–319 (311)	—	—	—
Cervical papillae†	431–476 (447)	—	422–524	post. to ep
Excretory pore†	394–420 (409)	—	384–486	300–480
Esophagus length†	675–712 (689)	600–700	541–620	540–620
Esophageal bulb width	176–274 (220)	180	—	130–260
Spicule length (mm)	1.24–1.46 (1.36)‡	—	1.00–1.42	1.00–1.42
Gubernaculum length	200–217 (207)	—	180–239	180–240
Dorsal ray length	469–994 (751)	—	420	740–860
Females				
Body length (mm)	9.15–12.4 (11.1)	12.0–12.5	10.5–12.8	9.10–12.8
Body width	441–566 (519)	500	—	480–670
Buccal capsule width	112–157 (130)	—	96–128	72–113
Buccal capsule depth	45–56 (49)	40	33–40	32–50
Elements of ELC	—	18–20	16–20	16–20
Elements of ILC	—	70–80	60–80	66–74
Nerve ring†	285–349 (317)	—	—	—
Cervical papillae†	439–547 (481)§	—	422–524	post. to ep
Excretory pore†	382–495 (476)	—	384–486	300–480
Esophagus length†	675–776 (733)	600–700	620–730	620–730
Esophageal bulb width	154–225 (220)	180	—	130–260
Vagina length	210–712 (356)§	—	—	—
Sphincter length	154–330 (228)	—	—	—
Infundibulum length	187–289 (228)	—	—	—
Vulva-to-anus length	79–180 (155)§	160	—	140–220
Female tail length	180–262 (214)§	140	140–193	140–240
Eggs (length × width)	67–92 (78) × 41–49 (46)	—	86–94 × 44–50	86–94 × 44–50

* Kotlan's measurements as reported by Theiler (1923).
† Measurement from anterior end.
‡ N = 3 of 4 males measured.
§ N = 6 of 7 females measured.

ronatus, which has a more uniform thickness overall (Figs. 19–22).

INSERTION OF ILC: The bases of the elements of the internal leaf crown rest, or are inserted, on the inner surface of the anterior part of the buccal capsule. In *C. sagittatus,* the ILC insertion is at a point about ⅓ of the depth of the buccal capsule (Figs. 19, 21); in *C. coronatus,* the ILC insertion is at ⅕ to ¼ of the buccal capsule depth (Figs. 20, 22).

RELATIVE WIDTH OF ELEMENTS OF ILC: A comparison of the width or thickness of the palisade-like elements of the ILC of the 2 species revealed that they are wider in *C. sagittatus* (Fig. 23) than they are in *C. coronatus* (Fig. 24).

ESOPHAGUS, NERVE RING, ANTERIOR DEIRIDS, EXCRETORY PORE: These characteristics are illustrated (Figs. 25, 26), and measurements pertaining to them are included in Tables 2 and 3, but differences between species were regarded as being related to differences in size of the nematodes. On average, *C. sagittatus* is 16–22% longer than *C. coronatus* (Tables 2, 3).

DORSAL RAY OF COPULATORY BURSA: Almost

Table 3. Morphometric comparison (in micrometers unless otherwise indicated, range and mean) of *Coronocyclus coronatus* with data reported by Theiler (1923), Dvojnos and Kharchenko (1994), and Hartwich (1994).

Characters	Present study	Theiler (1923)	Dvojnos and Kharchenko (1994)	Hartwich (1994)
Males				
Body length (mm)	8.42–9.32 (8.56)	8.75–9.00	6.84–10.28	6.40–10.3
Body width	300–370 (335)	340–360	—	210–410
Buccal capsule width	67–75 (69)	24–28	62–92	39–100
Buccal capsule depth	37–49 (43)	44–50	32–40	28–51
Elements of ELC	—	22	20–22	20–24
Elments of ILC	—	—	72–80	70–80
Nerve ring*	228–255 (244)	—	—	—
Cervical papillae*	317–379 (362)	280–300	320–376	250–440
Excretory pore*	284–356 (329)	280–300	—	250–440
Esophagus length	442–499 (454)	380–400	360–520	360–680
Esophageal bulb width	97–262 (133)	100	—	90–100
Spicule length (mm)	0.72–1.06 (0.94)	—	0.72–1.35	0.67–1.40
Gubernaculum length	146–169 (164)	—	156–210	110–200
Dorsal ray length	600–675 (648)	600	502–717	460–720
Females				
Body length (mm)	8.14–10.4 (9.06)	9.50–10.0	7.50–10.47	5.90–10.5
Body width	300–416 (374)	400–440	—	240–460
Buccal capsule width	65–75 (72)	24–28	68–126	39–100
Buccal capsule depth	39–45 (43)	44–50	38–44	28–51
Elements of ELC	—	22	20–22	20–24
Elements of ILC	—	—	72–80	70–80
Nerve ring*	244–281 (259)	—	—	—
Cervical papillae*	311–394 (362)	280–300	352–450	250–440
Excretory pore*	300–378 (329)	280–300	—	250–440
Esophagus length	469–510 (491)	380–400	400–683	360–680
Esophageal bulb width	105–146 (133)	100	—	180
Vagina length	247–435 (336)	280–360	280–360	—
Sphincter length	240–307 (260)	—	—	—
Infundibulum length	206–318 (251)	—	—	—
Vulva-to-anus length	112–120 (115)	120–140	74–89†	75–150
Female tail length	146–180 (164)	160–200	150–223	120–220
Eggs (length × width)	79–93 (84) × 37–55 (44)	80–90 × 36–44	92–103 × 44–55	75–103 × 36–55

* Measured from the anterior end.

† Calculated measurement from Dvojnos and Kharchenko (1994).

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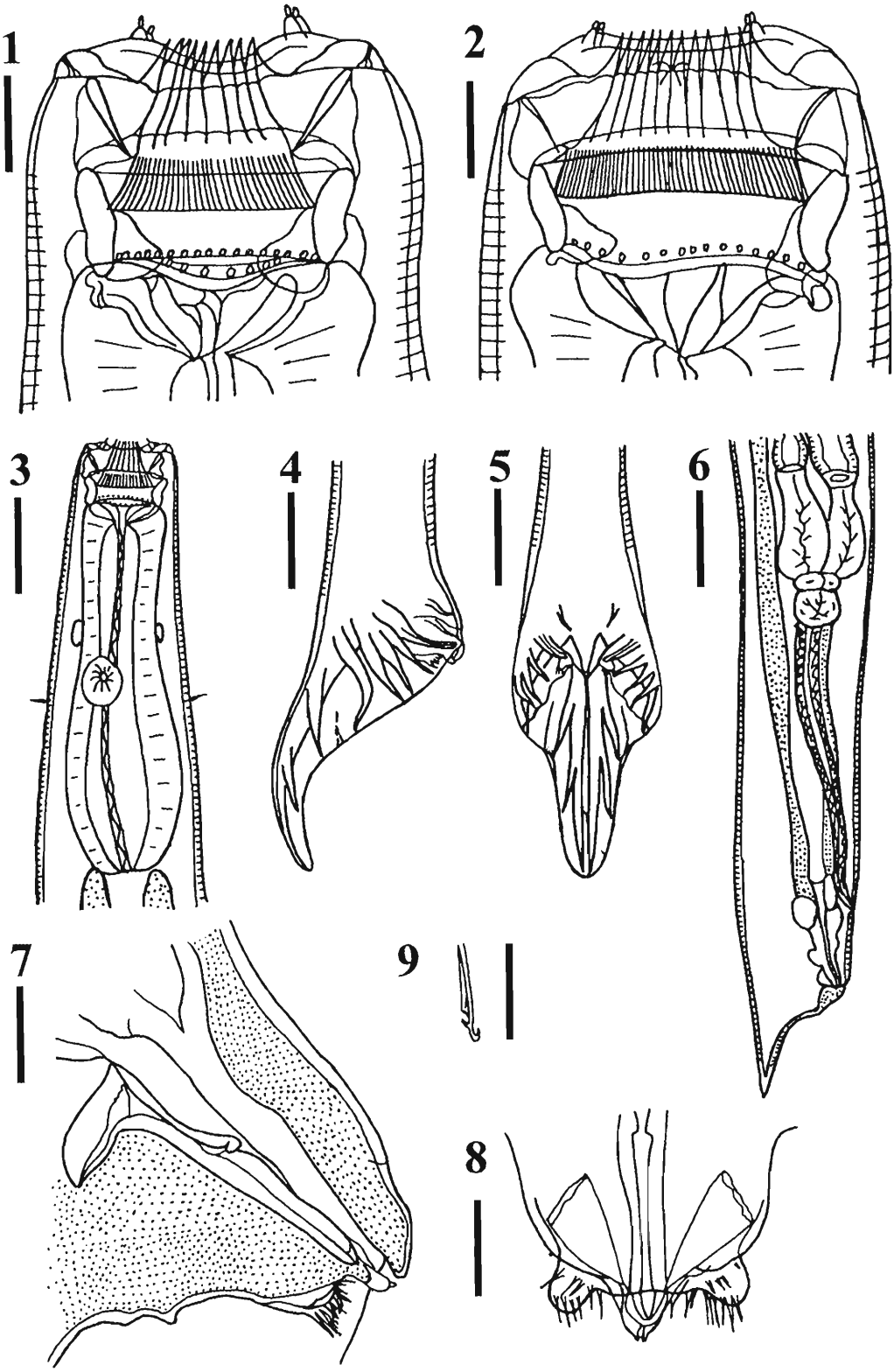
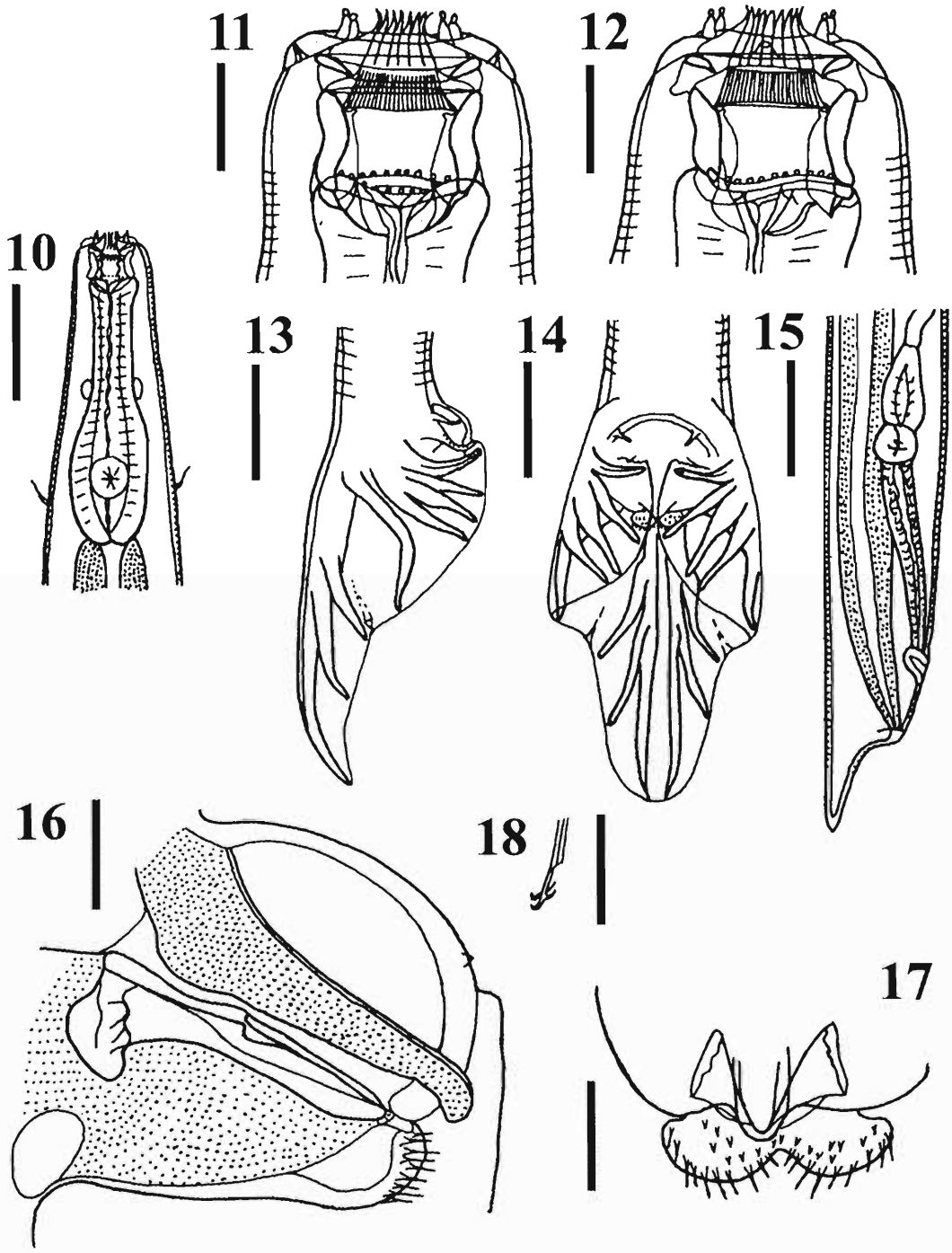
Figures 1–9. Camera lucida drawings of *Coronocyclus sagittatus*. Scale bars = 50 μm (Figs. 1, 2, 7–9) and 200 μm (Figs. 3–6). 1. Anterior end, dorsoventral view, showing optical section of cylindrical buccal capsule; a shallow, thick-walled esophageal funnel; elements of ILC; elements of ELC; spindle-shaped optical sections of ringlike sclerotized support (supports) for elements of ELC; mouth collar; lateral papillae (amphids); and 4 submedian cephalic papillae. 2. Anterior end, lateral view, showing same structures as in previous figure from different view. 3. Anterior end, ventral view, showing esophagus, nerve ring, anterior deirids, and excretory pore. 4. Male tail, lateral view. 5. Male tail, ventral view. 6. Female tail, lateral view, showing anus, vulva, and some portions of the ovejectors. 7. Genital cone of male, lateral view, showing gubernaculum and projections on ventral part of cone. 8. Genital cone, ventral view, showing distal tip of gubernaculum, internal plates, and projections of ventral part of cone. 9. Fused spicule tips.





Figures 10–18. Camera lucida drawings of *Coronocyclus coronatus*. Scale bars = 50 μm (Figs. 11, 12, 16–18) and 200 μm (Figs. 10, 13–15). 10. Anterior end, ventral view, showing esophagus, nerve ring, anterior deirids, and excretory pore. 11. Anterior end, dorsoventral view, showing optical section of cylindrical buccal capsule; a shallow, thick-walled esophageal funnel; elements of ILC; elements of ELC; spindle-shaped optical sections of ringlike sclerotized support (supports) for elements of ELC; mouth collar; lateral papillae (amphids); and 4 submedian cephalic papillae. 12. Anterior end, lateral view,

all dorsal rays of the Cyathostominea have 6 branches, 3 on each side of a medial fissure that usually extends to a point between the level of the origins of the proximal branches of the dorsal ray and the externodorsal rays. The 3 branches on each side of the dorsal ray are the proximal branch, the middle branch, and the longest or main branch (Figs. 4, 5, 13, 14, 27, 28). In *C. sagittatus,* the distance between the origin of the proximal branch and the origin of the medial branch is equal to or less than the length of the proximal branch. The proximal branch does not overlap the origin of the middle branch (Figs. 5, 27). In *C. coronatus,* the distance between the origins of the proximal and middle branches is less than the length of the proximal branch, and the proximal branch overlaps the origin of the middle branch (Figs. 14, 28). In *C. sagittatus,* the dorsal ray is divided by the medial fissure only to the level of the proximal branch, but in *C. coronatus* the medial fissure divides the dorsal ray to its base.

RELATIVE LENGTH OF FEMALE TAIL TO DISTANCE BETWEEN VULVA AND ANUS: Based on previously published measurements of *C. sagittatus* and *C. coronatus,* the length of the female tail compared to the distance between the vulva and the anus appeared to provide a useful character in females for distinguishing these 2 species. In *C. sagittatus,* these measurements were reported to be about equal, but in *C. coronatus,* the tail was reported to be longer than the distance from vulva to anus (Tables 2, 3). However, our measurements of just 6 females of *C. sagittatus* found the vulva-to-anus distance to be somewhat shorter than the tail length, as in *C. coronatus* (Table 2).

Discussion

The characters described in the Results section and illustrated in the photomicrographs and line drawings can be used to distinguish adult, fifth-stage specimens of the 2 similar species *Coronocyclus coronatus* and *C. sagittatus.* Three key cephalic characters that are applicable to both sexes (the shape of the buccal capsule, the shape of the buccal capsule wall in optical section, and the point of insertion of elements of the ILC on the inside of the buccal capsule wall) are provided, and an additional key character is provided for males (the configuration of the branches of the dorsal ray of the copulatory bursa of the male).

Our data indicated that characteristics of the female tail could not be used to separate these species. Previously published measurements indicated that differences between the distance from vulva to anus and the length of the tails and differences between vagina lengths might exist between *C. sagittatus* and *C. coronatus* (Tables 2, 3), but our measurements did not support the use of these characters. Our method of sampling variation within species involved measuring a few specimens from as many different isolates as was practical. We included specimens from 3 different isolates of each species in our measurements. This may explain the greater range of variation in our measurements compared to those described in earlier reports (Tables 2, 3).

Although the greater width of the ILC elements of *C. sagittatus* can be used as a supplementary character to distinguish the species from *C. coronatus,* the numbers of elements in the leaf crowns cannot. Because of overlap and considerable variation in the number of elements in the ELC and in the ILC, the number of elements is not a useful character for separating these species. Kosupko and Nechinenny (1982) studied en face preparations of both species and reported on *C. sagittatus* as having 20 ELC elements and 68–74 ILC elements. They reported that *C. coronatus* had 20–22 ELC elements and 72–80 ILC elements. Braide and Georgi (1974) studied en face preparations of *C. coronatus* and reported 22 ELC elements. Looss (1900, 1902) reported "about 22" ELC elements in *C. coronatus.* In his survey of the literature, Hartwich (1994) reported a wide range of numbers of leaf crown elements.

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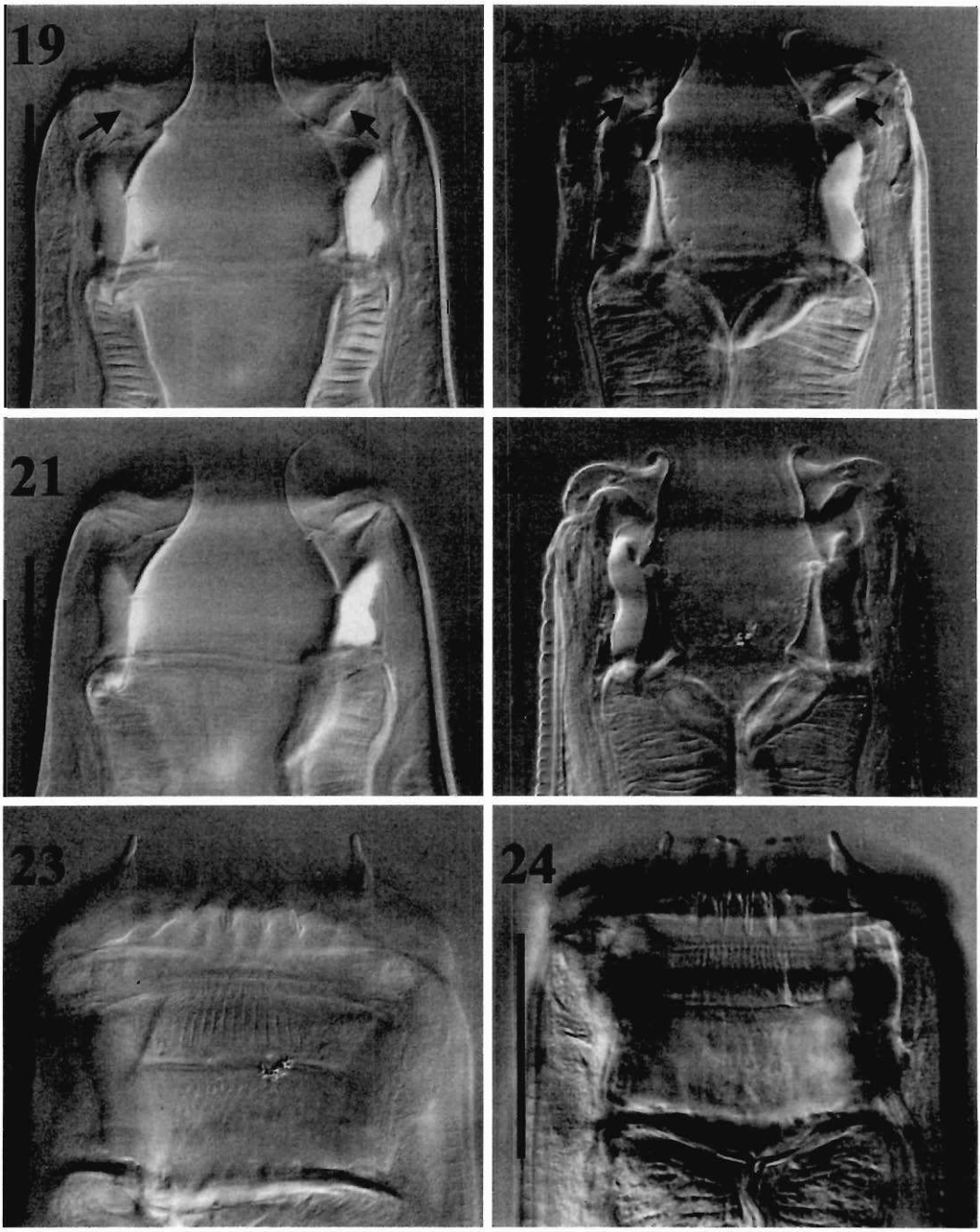
showing same structures as in previous figure from different view. 13. Male tail, lateral view. 14. Male tail, ventral view. 15. Female tail, lateral view, showing anus, vulva, and some portions of the ovejectors. 16. Genital cone of male, lateral view, showing gubernaculum and projections on ventral part of cone. 17. Genital cone, ventral view, showing distal tip of gubernaculum, internal plates, and projections of ventral part of cone. 18. Fused spicule tips.



Figures 19–24. ***Coronocyclus sagittatus*** **and** ***C. coronatus,*** **photomicrographs of key differentiating characters. Scale bars = 50 μm. Figures 19, 20. Anterior ends, dorsoventral views, showing optical sections of cylindrical buccal capsules, elements of ILC and ELC, spindle-shaped optical sections of ringlike sclerotized supports of the ELC (supports) (arrows), and lateral papillae (amphids). 19.** ***C. sagittatus.*** **20.** ***C. coronatus.*** **Figures 21, 22. Anterior ends, lateral views, showing optical sections of cylindrical buccal capsules, elements of ILC and ELC, spindle-shaped supports, and the mouth collar. 21.** ***C. sagittatus.*** **22.** ***C. coronatus.*** **Figures 23, 24. Anterior ends, dorsoventral views, focused on submedian cephalic papillae and elements of the ILC. 23.** ***C. sagittatus.*** **24.** ***C. coronatus.***

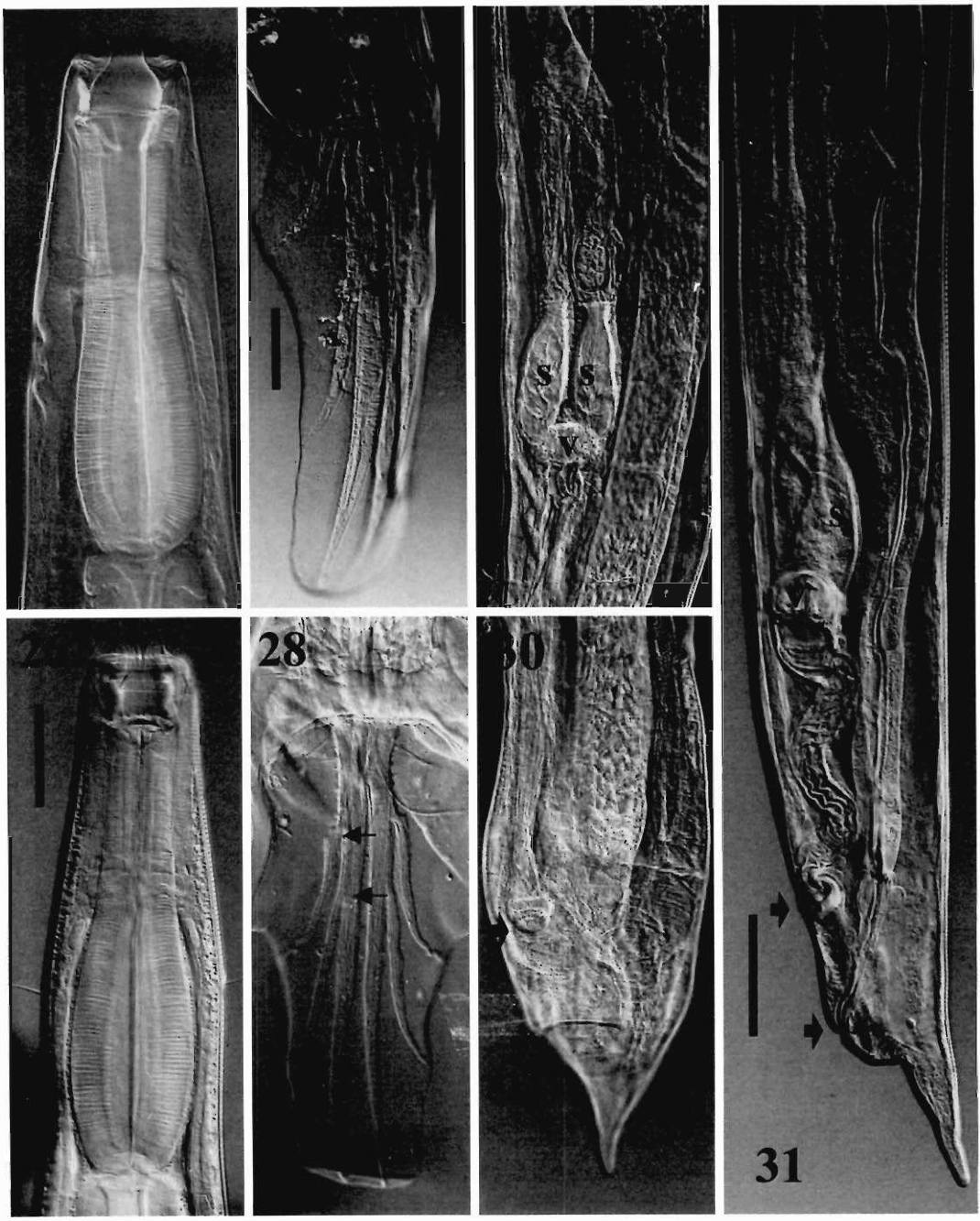


Figure 25–31. *C. sagittatus* and *C. coronatus,* photomicrographs of the esophagus and key differentiating characters of the male and the female. Scale bars = 100 μm. 25. *C. sagittatus,* lateral view (arrow at excretory pore). 26. *C. coronatus,* dorsoventral view, showing positions of nerve ring and cervical papillae (anterior deirids). Figures 27, 28. Male tails, dorsolateral and dorsal views respectively, showing proximal (upper arrow), middle (lower arrow), and main branches of the dorsal rays. 27. *C. sagittatus.* 28. *C. coronatus.* Figures 29–31. Female tails, showing positions of anus, vulva (large arrows), and constituent parts of the ovejectors including vestibule (v), sphincters (s), and infundibula (between small arrows). 29, 30. *C. sagittatus.* 31. *C. coronatus.*

Distinguishing *C. sagittatus* from *C. coronatus* requires a careful comparison of the characteristics of the buccal capsules and of the ILC elements. To assist in these identifications, we have provided photomicrographs of these characteristics side by side (Figs. 19–24). This improved identification aid should provide the research tools needed to determine whether the apparently low distribution of the rarely reported *C. sagittatus* has resulted from an inability to distinguish it from the more commonly reported *C. coronatus,* as suspected by Hartwich (1994).

Coronocyclus coronatus is distributed throughout the range of the hosts, *Equus caballus, E. przhewalskii, E. asinus, E. hemionus,* and hybrids of horses. Its most common habitat is the caecum. *Coronocyclus sagittatus* is a rare species, not found in *E. asinus* or *E. hemionus.* Its preferred habitat is not known.

Larvae of the fourth stage have only been identified in *C. coronatus.* The larva's buccal capsule is large; the width is less than or equal to the length; and the walls are thick, with a sharply pointed anterior edge. A thick, triangular, ringlike anterior part of the lining of the esophageal funnel supports the buccal capsule. The esophageal funnel is well developed, with a pointed dorsal tooth that has a wide base. The tooth does not project into the buccal cavity (Dvojnos and Kharchenko, 1987).

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